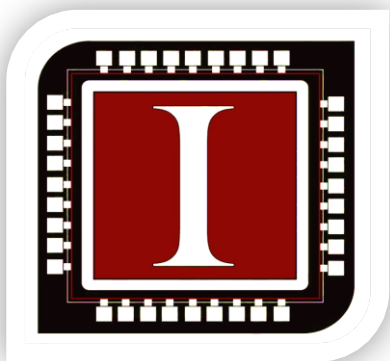


CMOS Biochips for Point-of-Care Molecular Diagnostics

Arjang Hassibi

InSilixa, Inc.

Sunnyvale, CA USA

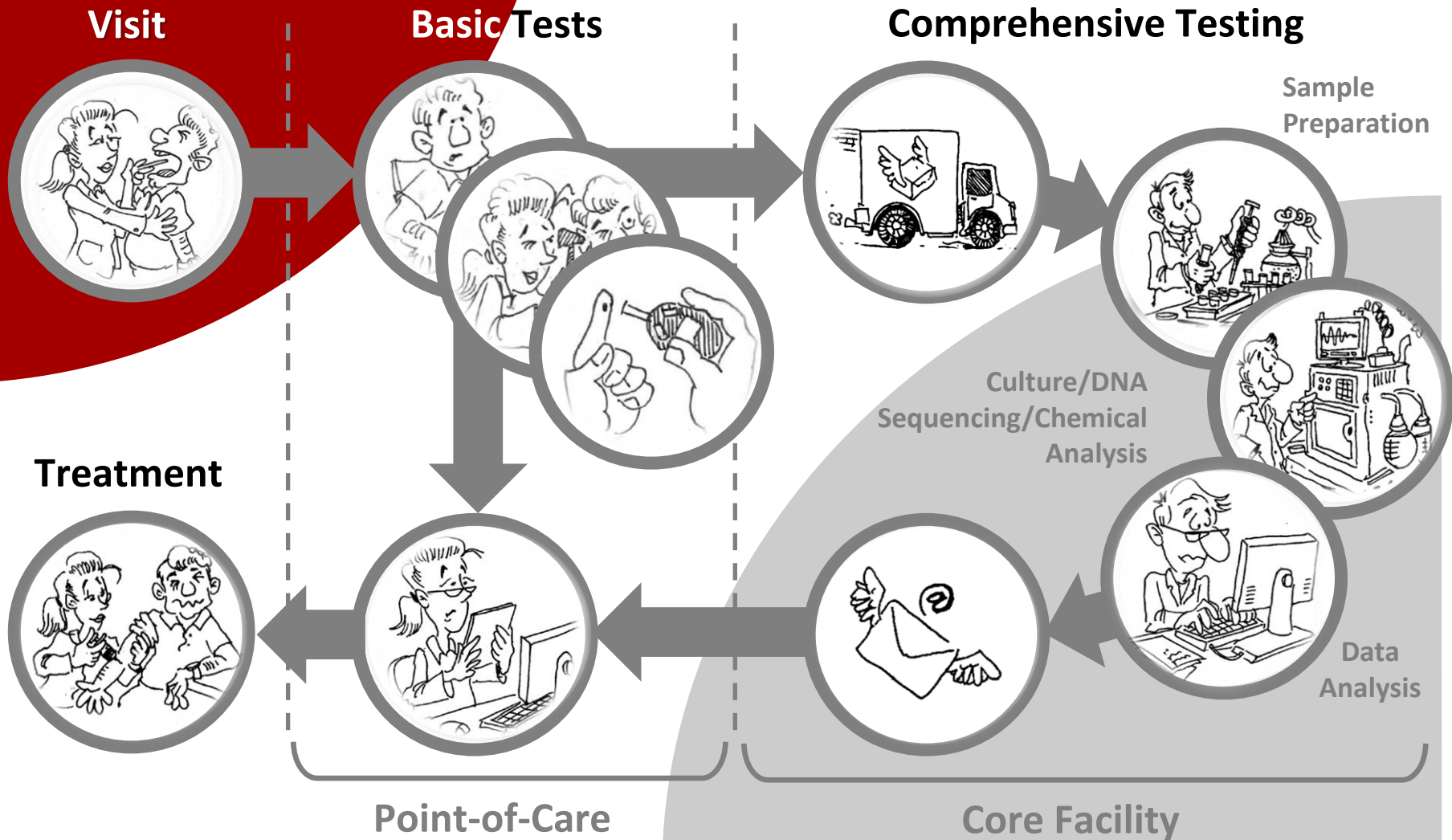


OVERVIEW

Molecular Diagnostics (MDx) and Stuff...

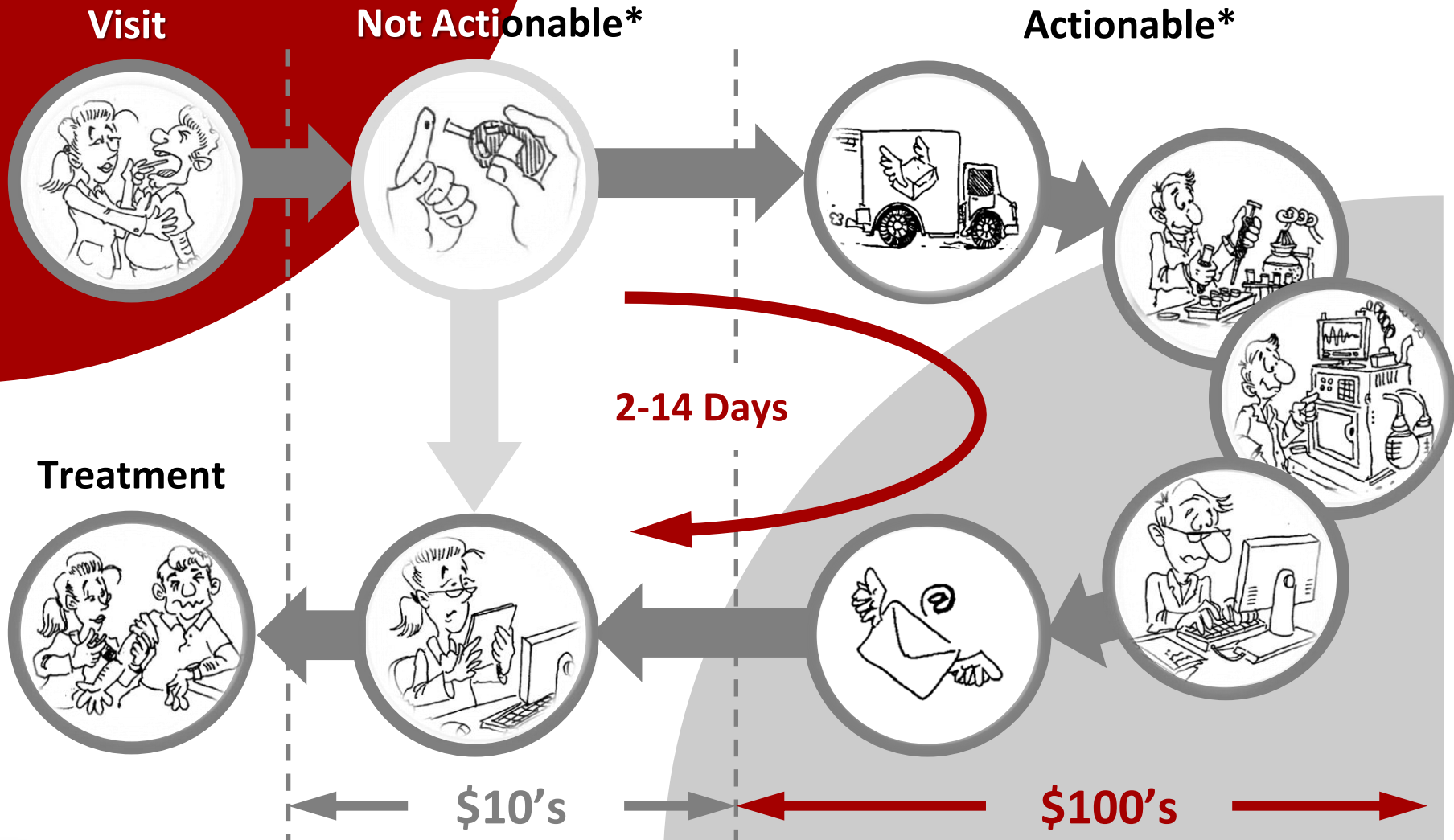
Diagnostics Information Flow (1)

Treatment options are based on diagnostics results



Diagnostics Information Flow (2)

Actionable tests* take too long and are very expensive



Molecular Diagnostics (MDx)

Highly actionable, but not mass-deployable

Urinary Tract Infection
(7M Visits/Year)



0.5 Hour

Dipstick Analyte
Test(\$30)

Infection
(67% Accuracy)

2 Days

Culture
(\$40-\$100)

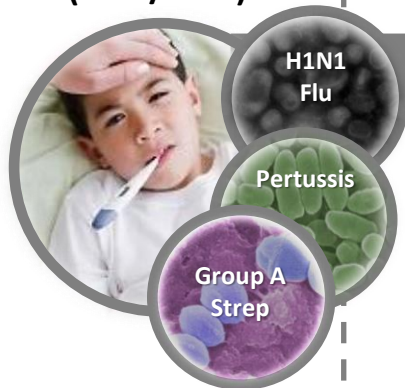
E-Coli Present
(+99% Accuracy)

14 Days

DNA Analysis
(>\$500)

E-Coli Present
Antibiotic Resistant Strain
(99% Accuracy)

Respiratory Infection
(40M/Year)



0.5 Hour

Throat Swab
(\$40)

Group A Strep Present
(73% Accurate)

2 Days

Culture
(\$40-\$100)

Group A Strep Present
(99% Accurate)

14 Days

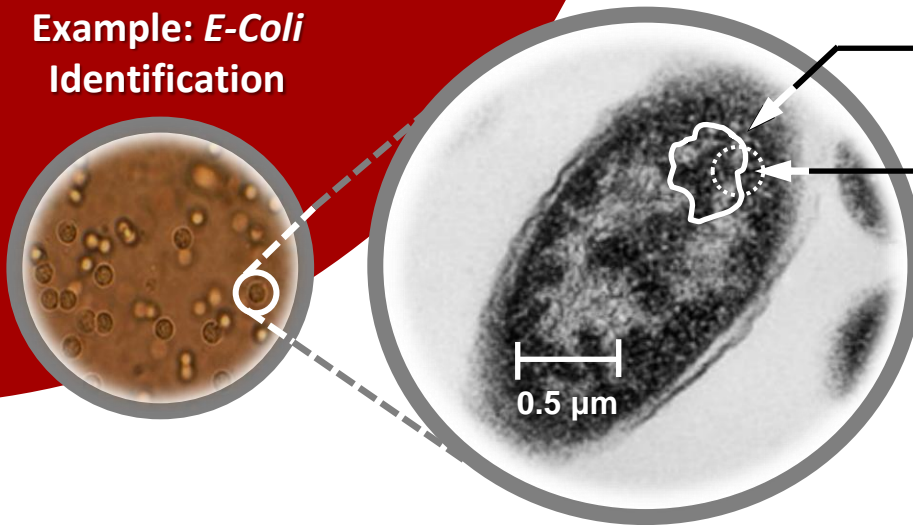
DNA Analysis
(>\$500)

Group A Strep, H1N1 Flu,
Pertussis, ...
(99% Accuracy)

MDx Problem Statement (1)

Identify unique molecular structures (e.g., DNA sequences) in presence of similar structures in a “dirty” biological environment

Example: *E-Coli*
Identification



E-Coli Genome (47 Million bases)

```
....CGGCGTTCGGCGGGGTTTTCCCGAG  
GTTCGGGGCGCCAAAAACGTTCTTCA  
AAAAGTAAGGTAACGTTTACGTTTCGA  
TTCAGGTTTCAGGTTTGGTTTAAAA  
GGTACCGTTTACAAAGGTATGGAAA...
```

A unique sequence found only in pathogenic *E-Coli*

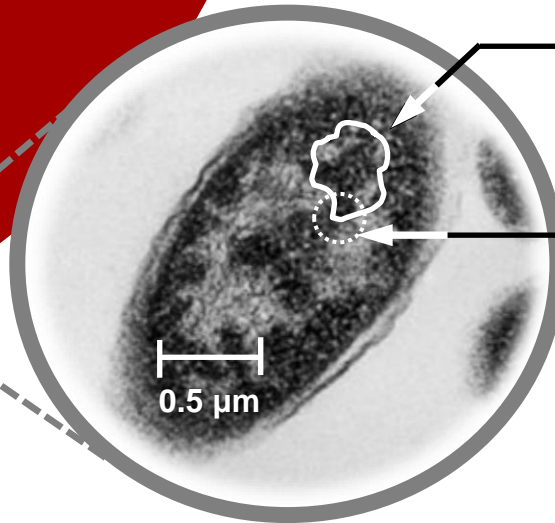
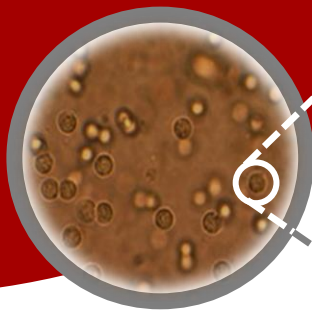
GTTTCGATTCAGGTTTCAGGTTTGGTT (28 bases)

$$\text{Prob \{Random Occurrence\}} = \left(\frac{1}{4}\right)^N \Big|_{N=28} \approx 1.388 \times 10^{-17}$$

MDx Problem Statement (2)

Check specific DNA sequence for random mutations that result in functional changes in the behavior of the organism

Example: *E-Coli*
Identification



E-Coli Genome (47 Million bases)

.... GTCTTAGGCAGTTAAA
CGTTTACGATAAACACGGT
ACGACGATTTTACAGGG...

Resistant to Antibiotic (Super Bug)

.... GTCTTAGGCAGTTAAA
CGTTTACGATGAACACGGT
ACGACGATTTTACAGGG...

Sensitive to Antibiotic (Normal Bug)

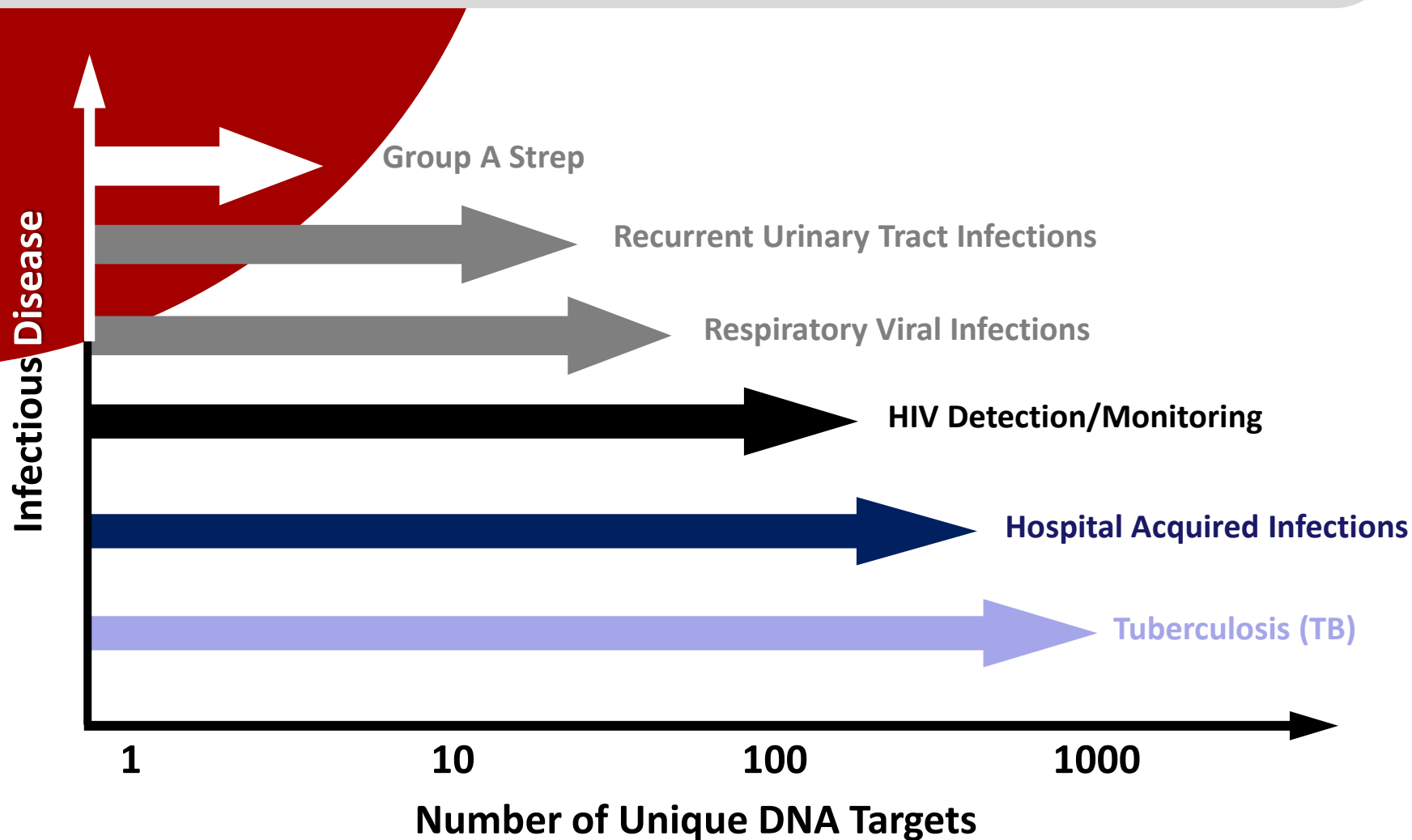
.... GTCTTAGGCAGTTAAA
CGTTTACGATAAACACGGT
ACGACGATTTTACAGGG...

← TGA ← TAA ←

Mutation

MDx Problem Statement (3)

For actionable infectious diseases MDx, 10's to 100's of unique DNA sequences and/or mutations should be detected

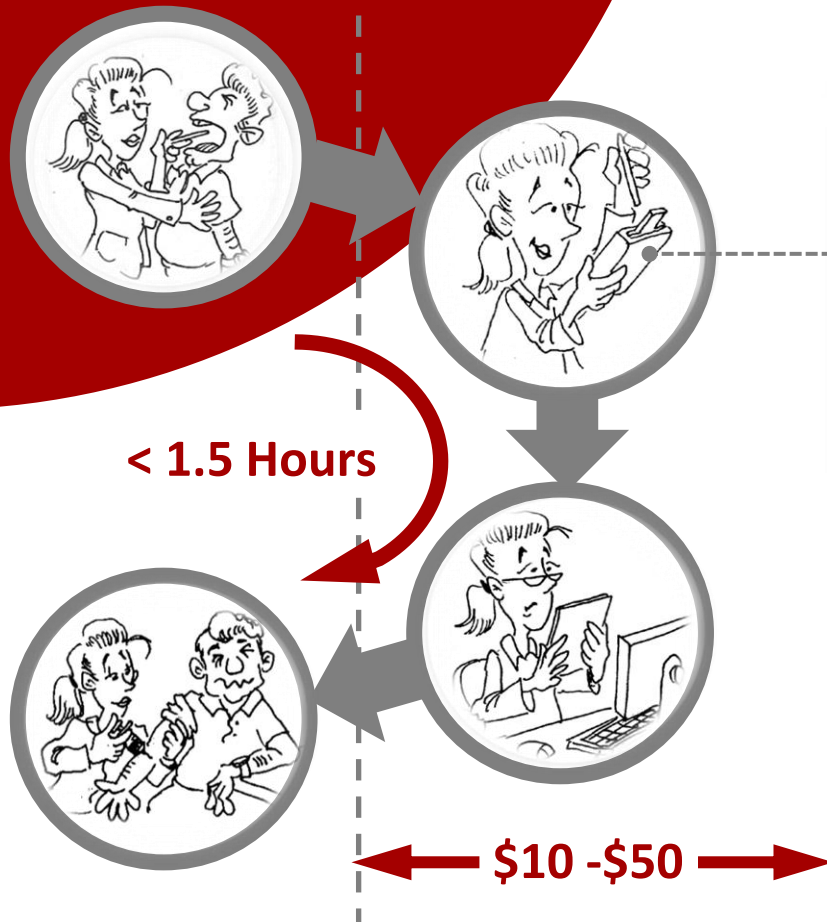


Current State-of-the-Art

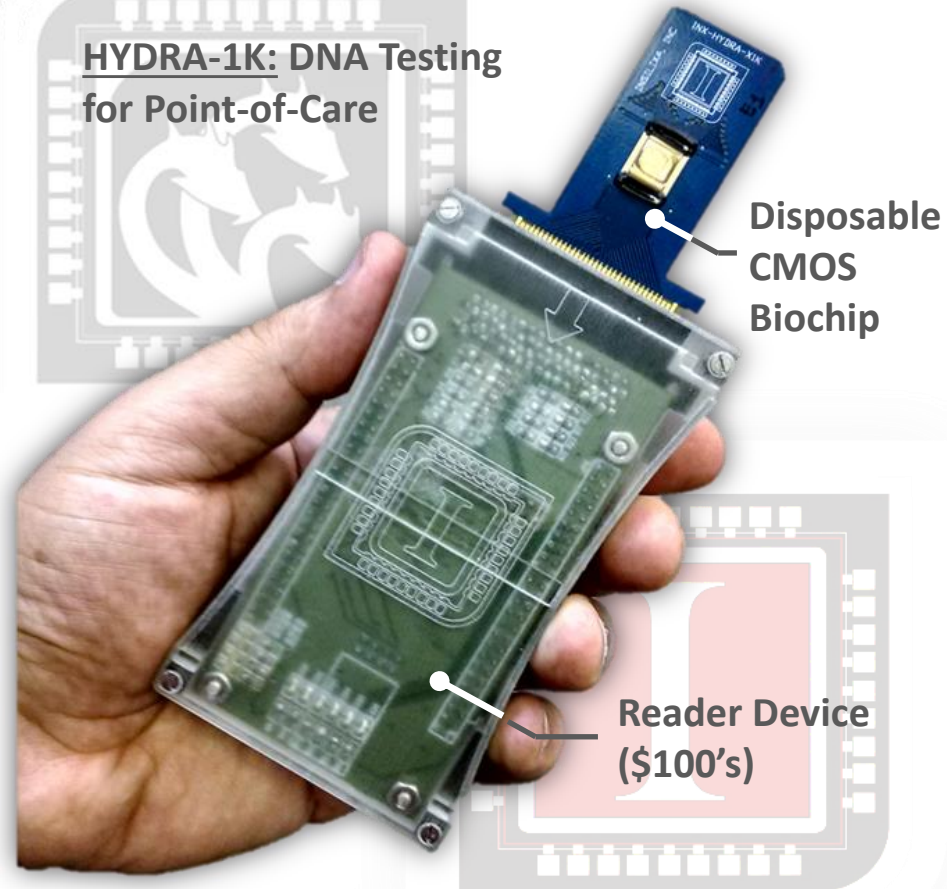
TECHNOLOGY	PCR*	DNA ARRAYS	DNA SEQUENCING	CURRENT NEED
Instrument (Setup) Price	\$20K -50K	\$25K-75K	\$100K-\$700K	< \$1000
Price per Test	\$80-\$400	\$200-\$1000	\$2.5K-\$10K	< \$50
Max DNA Targets	6-20	20-1000	+1M	1000
Detection Accuracy	High	Low	Medium	High
Test Time	2-3 hours	6-12 Hours	> 1 Day	1 Hour
Fully-Automated	Yes	No	No	Yes
Portable	No	No	No	Yes
PoC Compatible	Yes	No	No	Yes

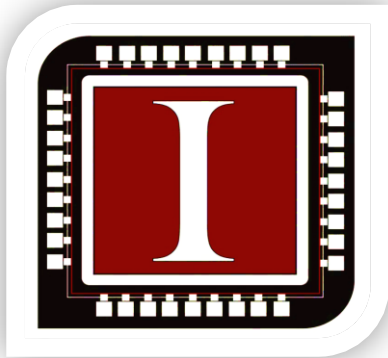
Solution: InSilixa's HYDRA-1K

Rapid (<1hr), low-cost (\$10's), and simple (sample-to-answer) MDx to detect up to 1000 Unique DNA Sequences



HYDRA-1K: DNA Testing
for Point-of-Care





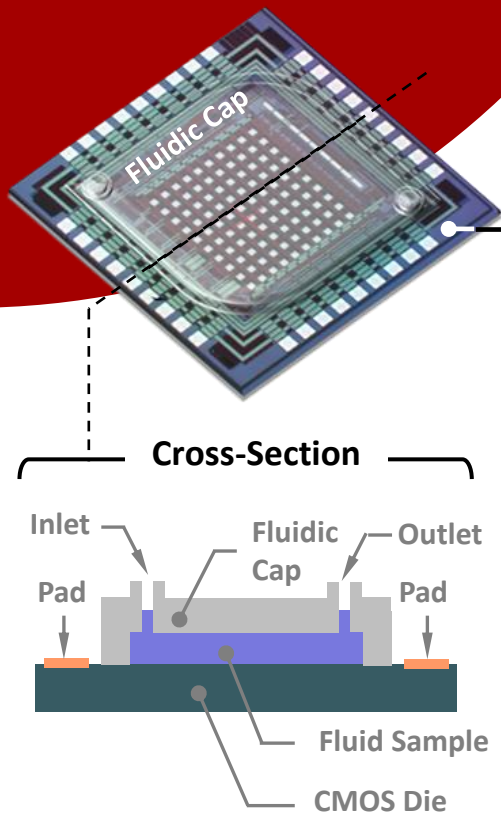
TECHNOLOGY

HYDRA-1K System

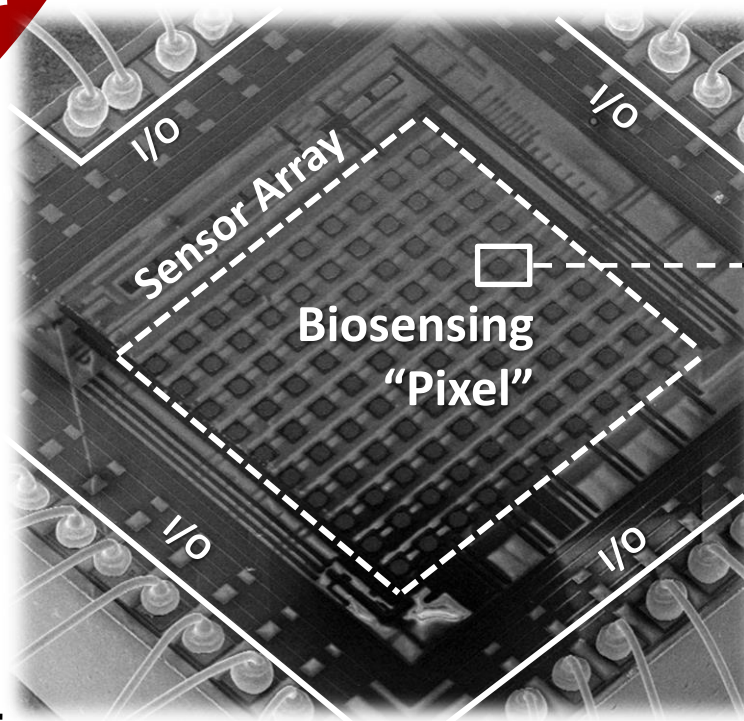
CMOS Biochips

CMOS ICs that are enhanced and specifically packaged to function as high-performance molecular sensors

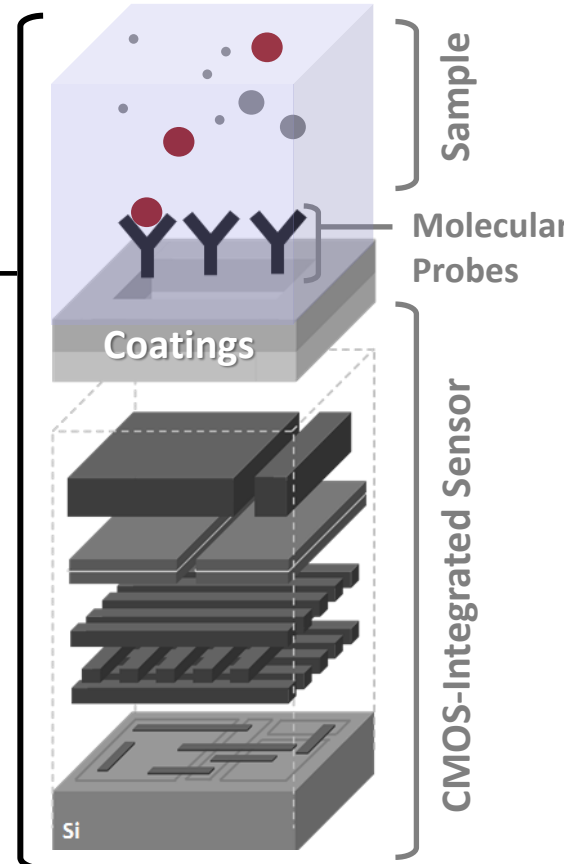
Biochip Module



CMOS Die



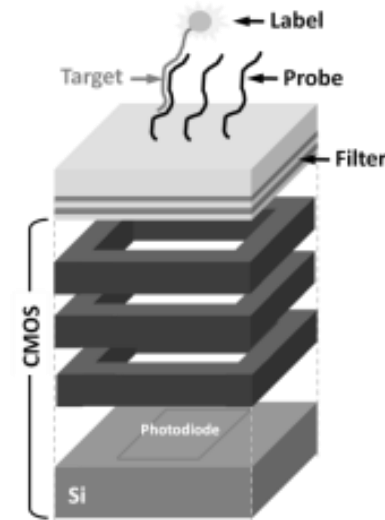
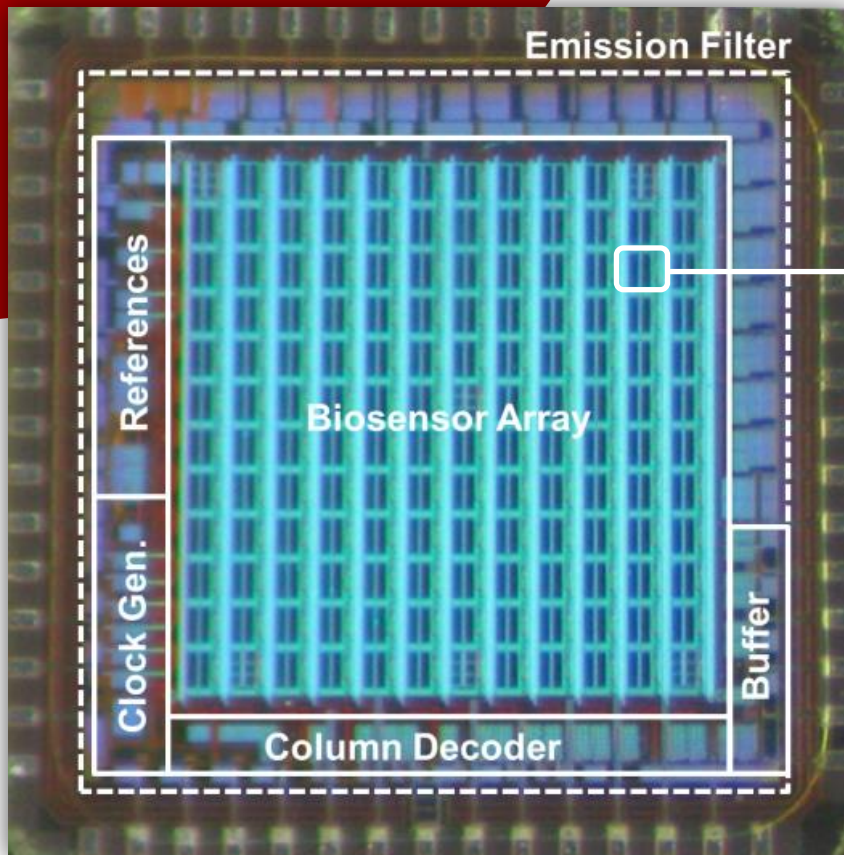
Biosensing Pixel



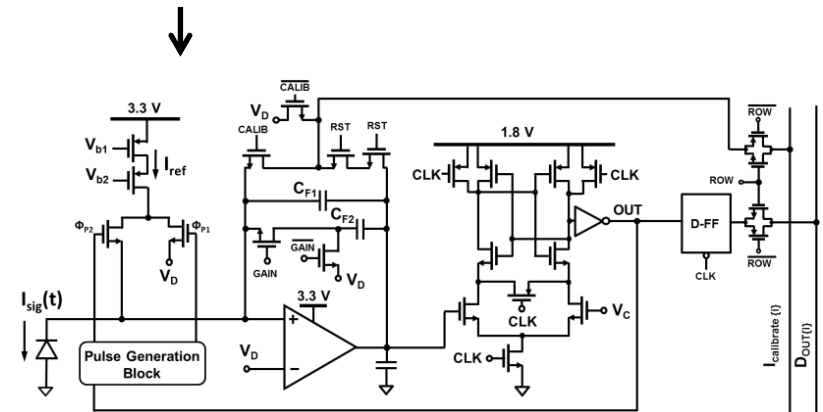
Biosensing Pixels

Depending on the adopted chemistry, various detection modalities can be implemented in the pixels

Fluorescent Detection Arrays*



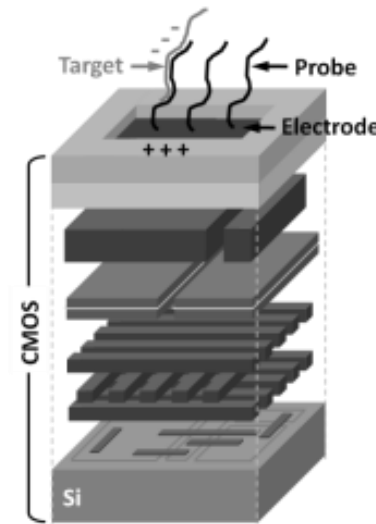
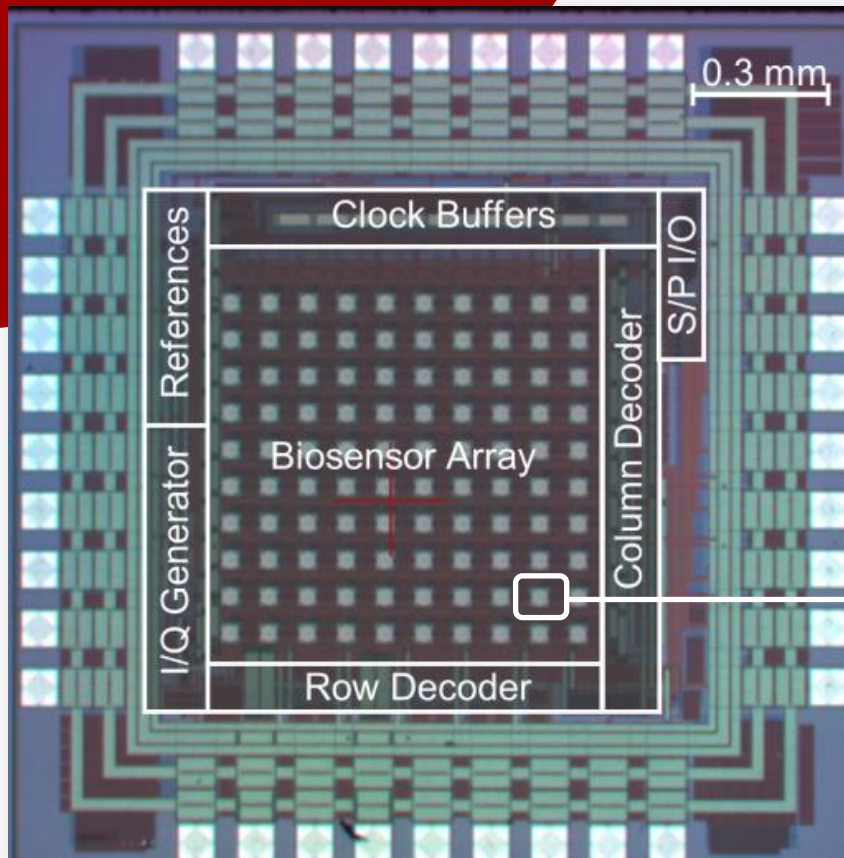
High dynamic range (HDR) wavelength-specific $\Delta\Sigma$ photo-sensor in every pixel



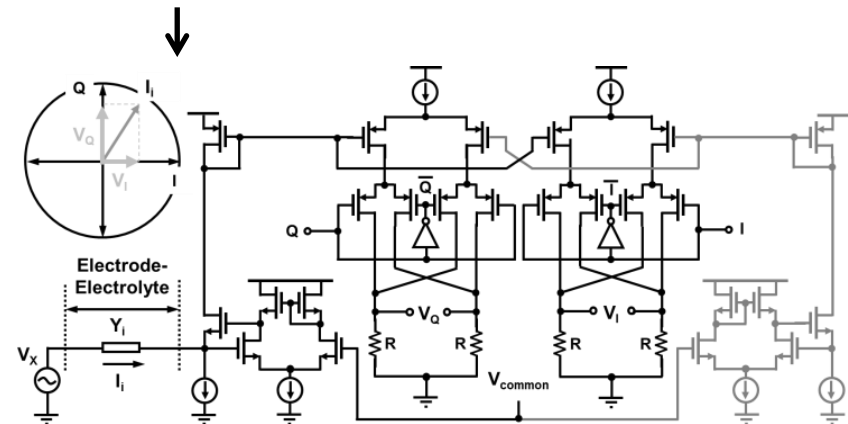
Biosensing Pixels

Depending on the adopted chemistry, various detection modalities can be implemented in the pixels

Impedance Spectroscopy Arrays*



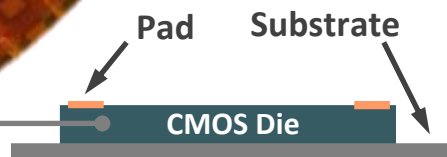
Lock-in amplifier-based impedance measurement in every pixel



Manufacturing Process

Biochips require a complex packaging/assembly process

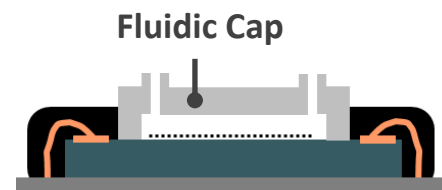
1 CMOS Wafers



2 Dicing and Mounting



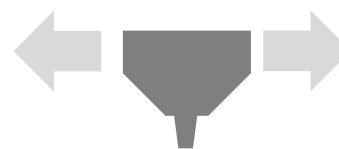
3 Wire-bonding



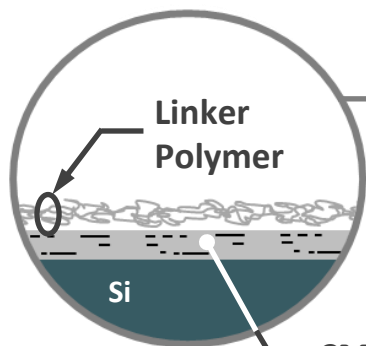
7 Fluidic Cap Assembly



6 Wash and Surface Block



5 Probe Spotting



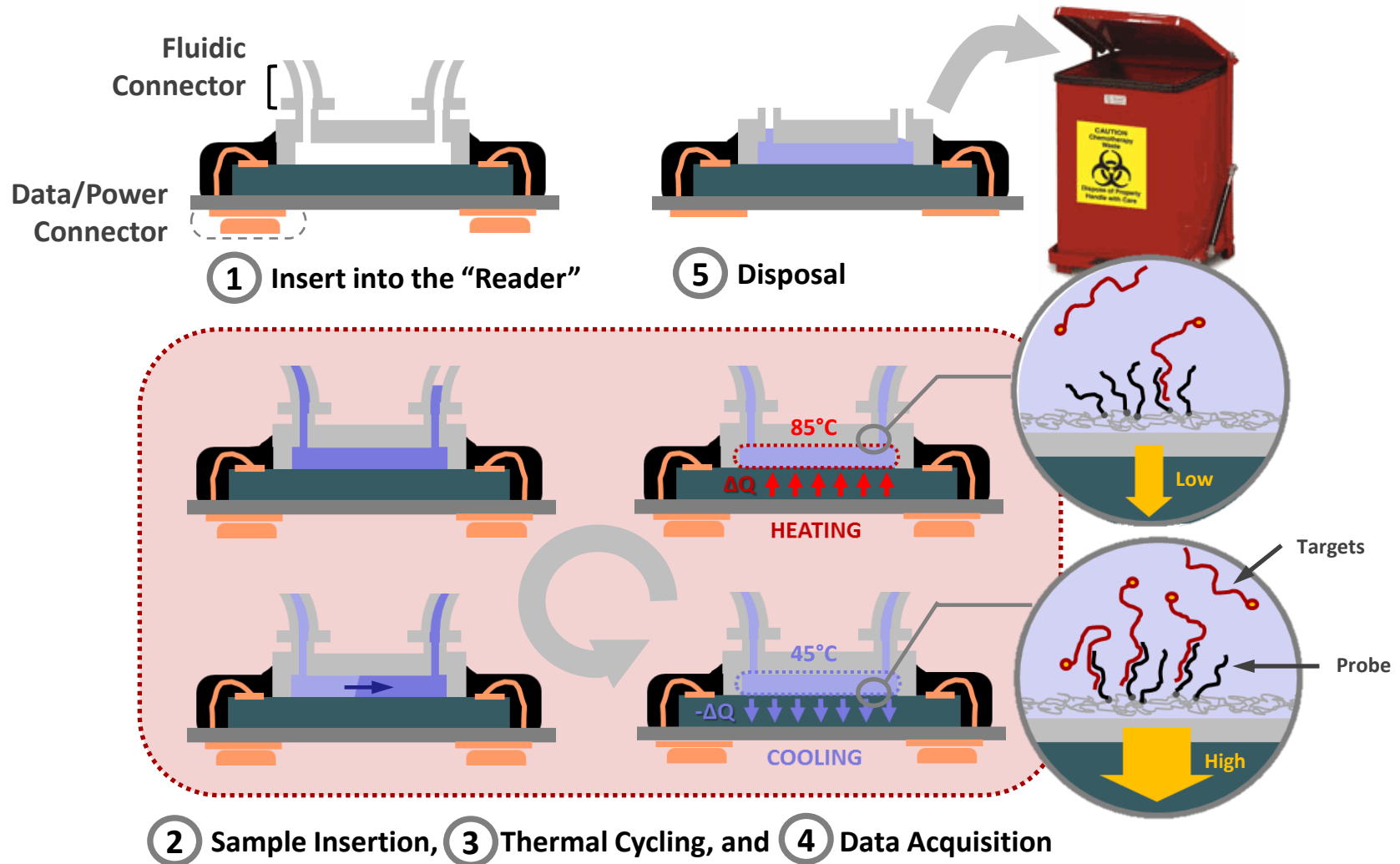
4 Surface Coating

CMOS Backend



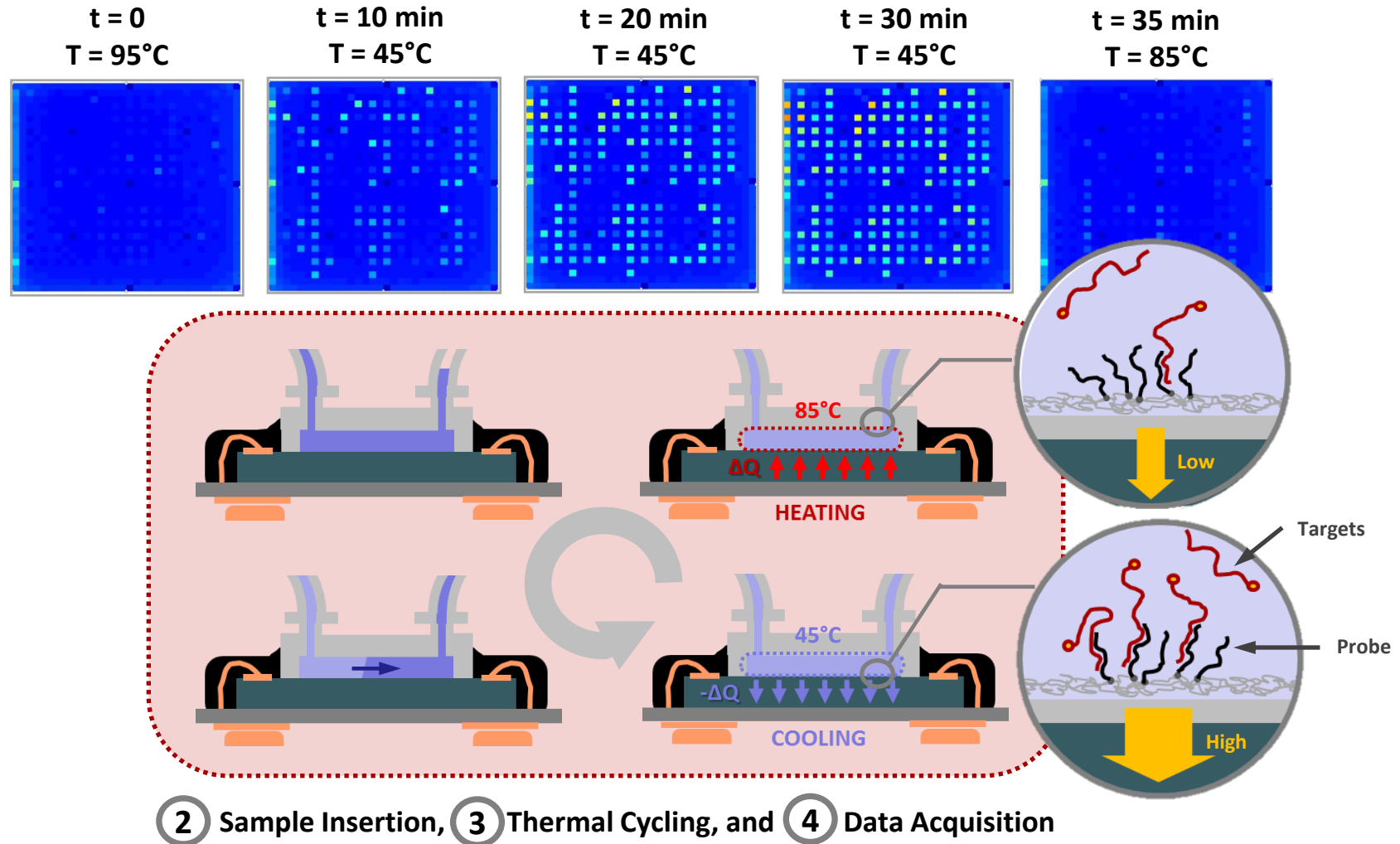
Detection Process (1)

Chip (and the pixels) are exposed to the sample containing the targets



Detection Process (2)

Output data report the DNA capturing events, as a function of time and temperature, in “every pixel”

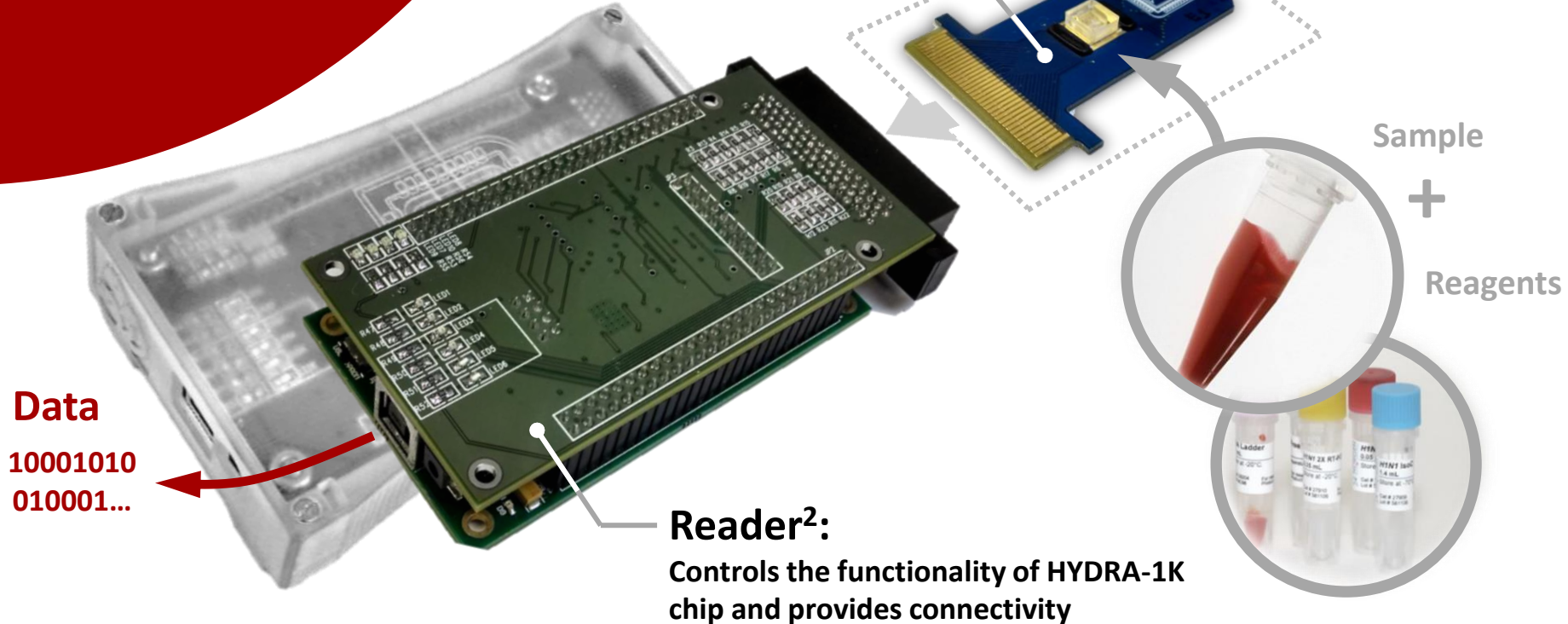


HYDRA-1K Platform

A CMOS biochip system for point-of-care DNA analysis

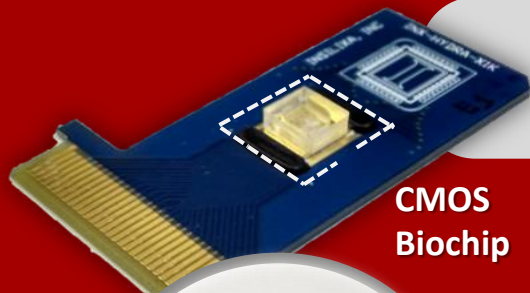
HYDRA-1K Biochip Module (\$7-\$15)¹:

Disposable module that electronically detects up to 1000 (1K) unique DNA sequences and provides a digital output.



HYDRA-1K: Open Platform for MDx

HYDRA-1K reagents, hardware, and software are designed to enable flexible application development



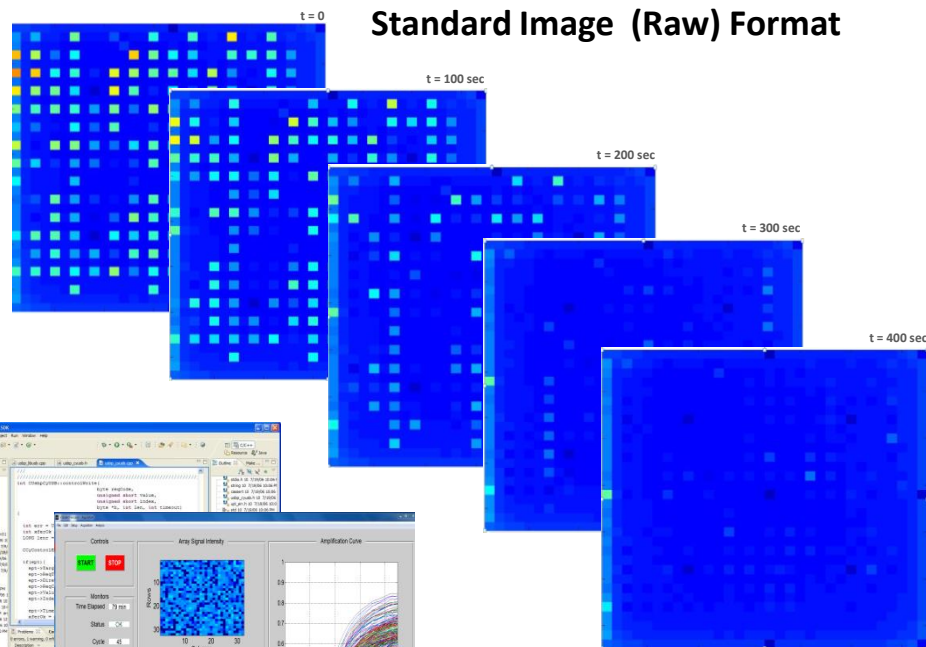
CMOS
Biochip



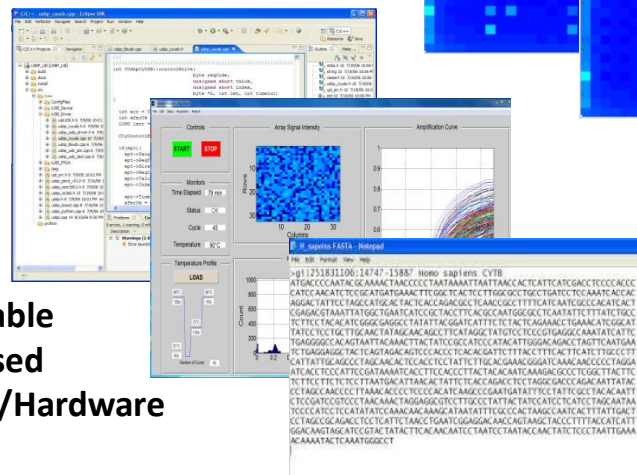
Standard
Reagents and
DNA Probes



Configurable
FPGA-based
Software/Hardware



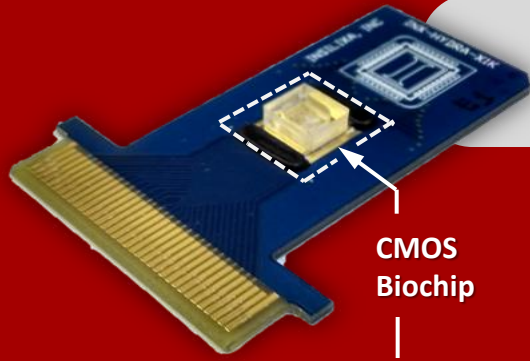
Standard Image (Raw) Format



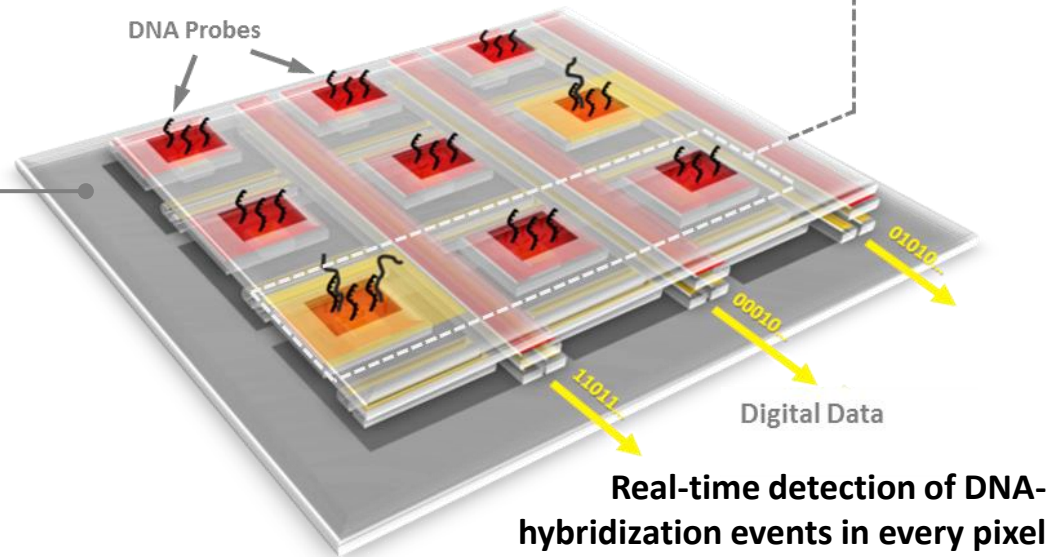
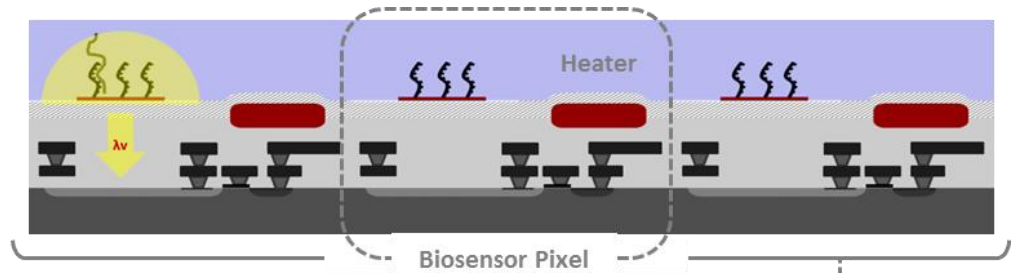
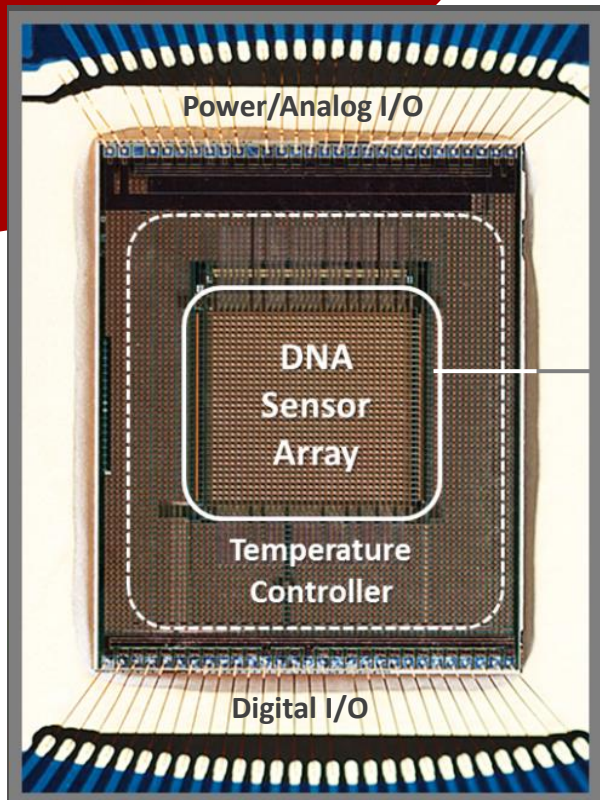
Standard (FASTA)
Output File

HYDRA-1K: DNA Sensor Array

DNA sequence identification enabled by pixel-level DNA capturing and optical detection

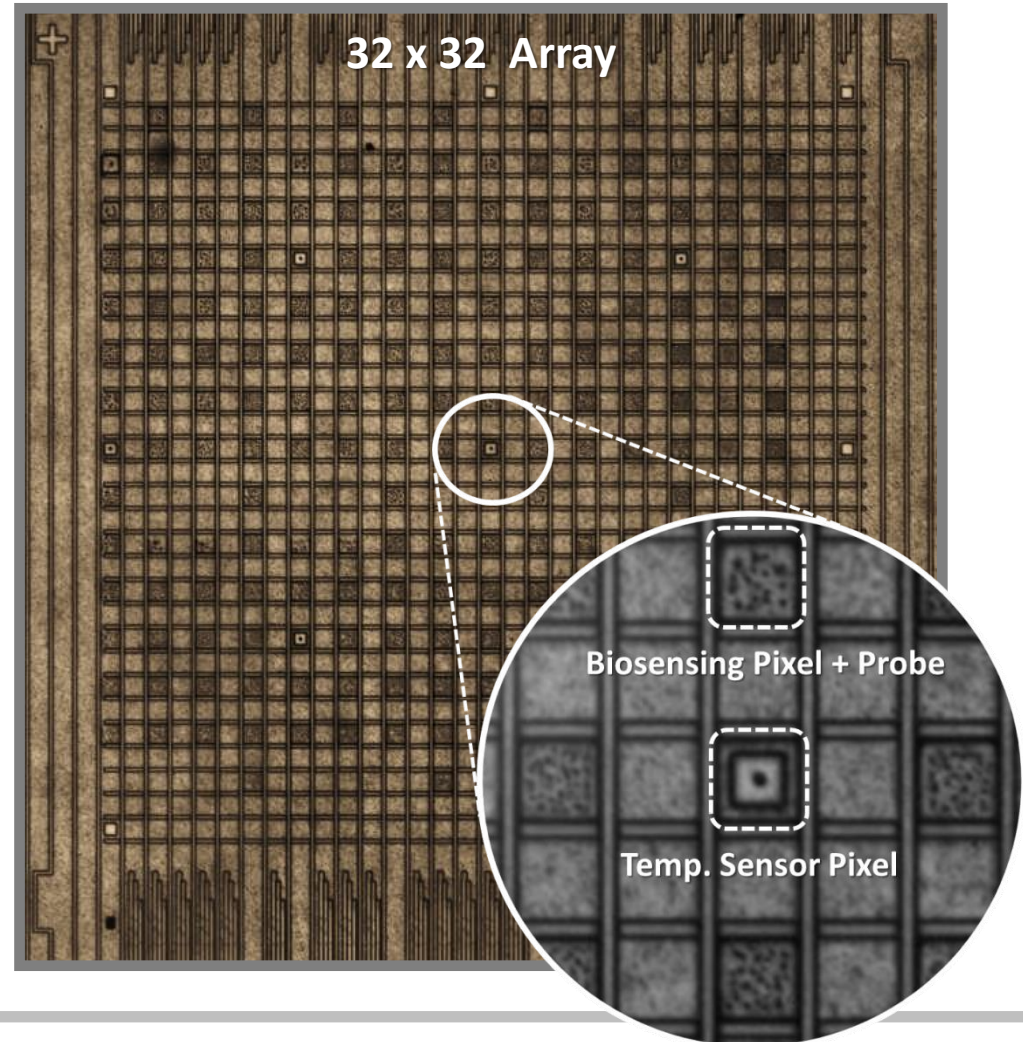
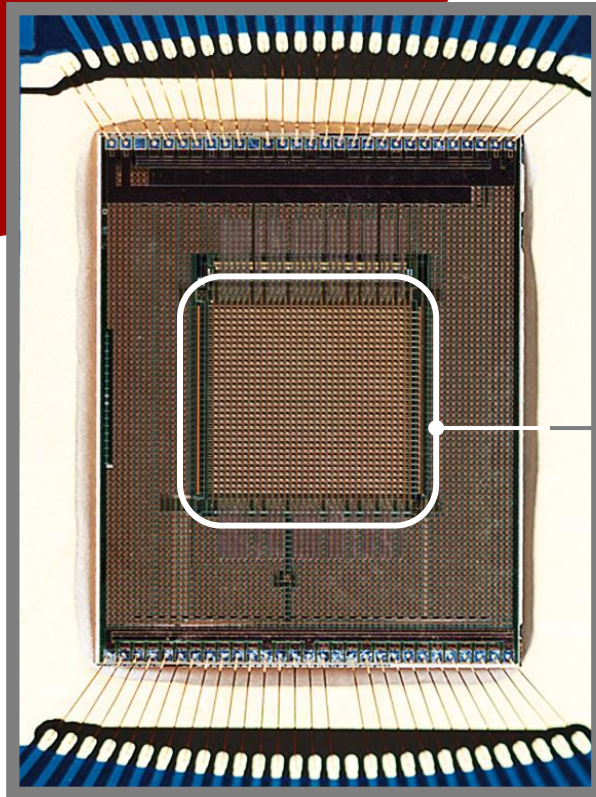
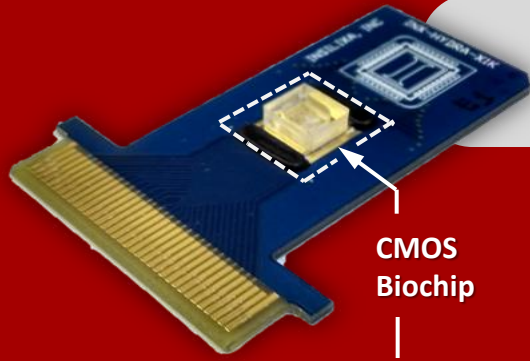


CMOS
Biochip



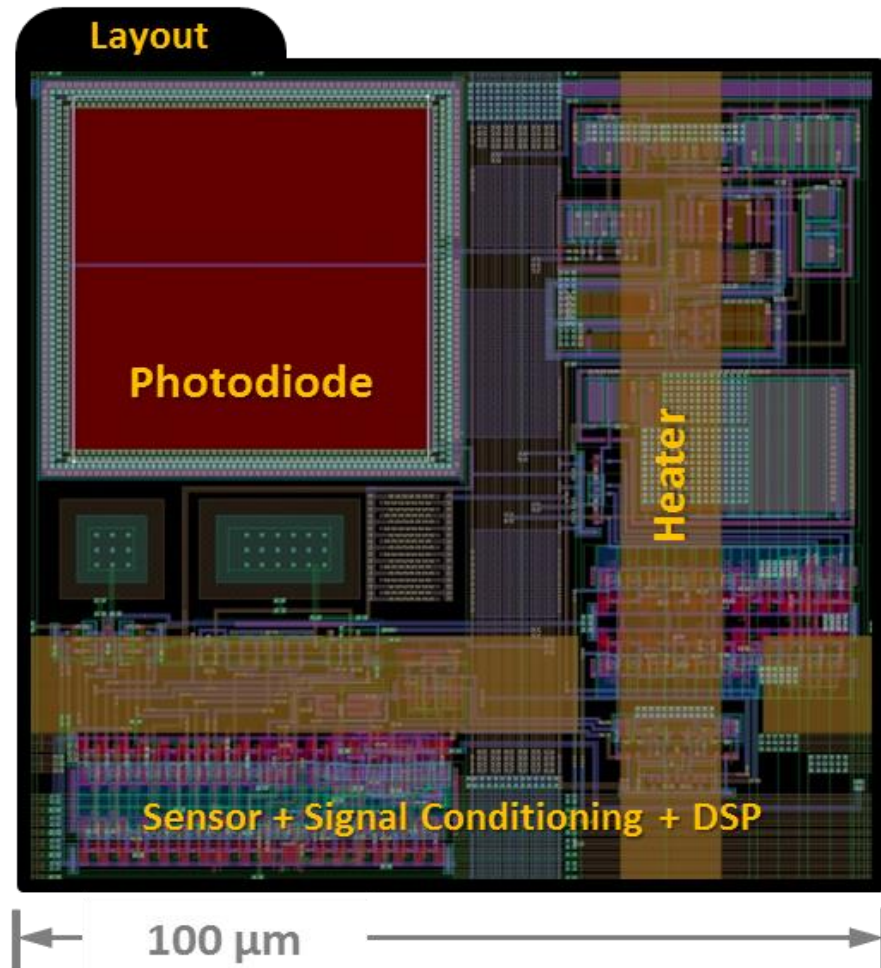
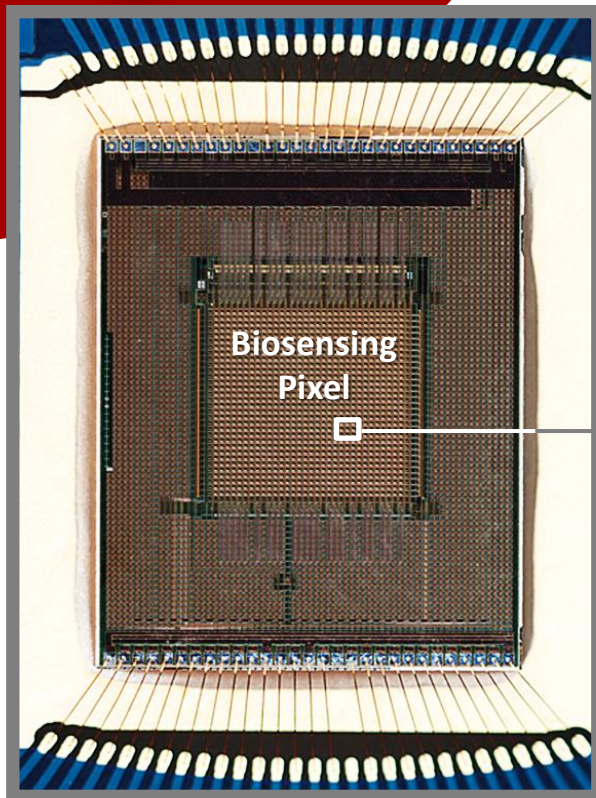
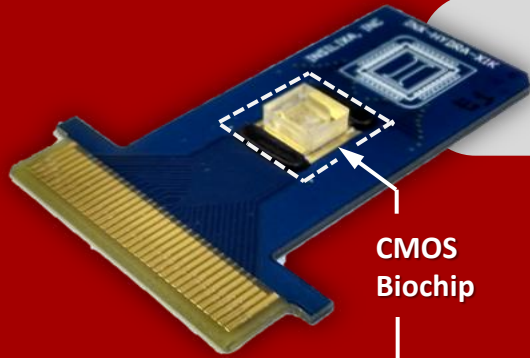
HYDRA-1K: CMOS Biosensor Array

DNA sequence identification enabled by pixel-level DNA capturing and optical detection



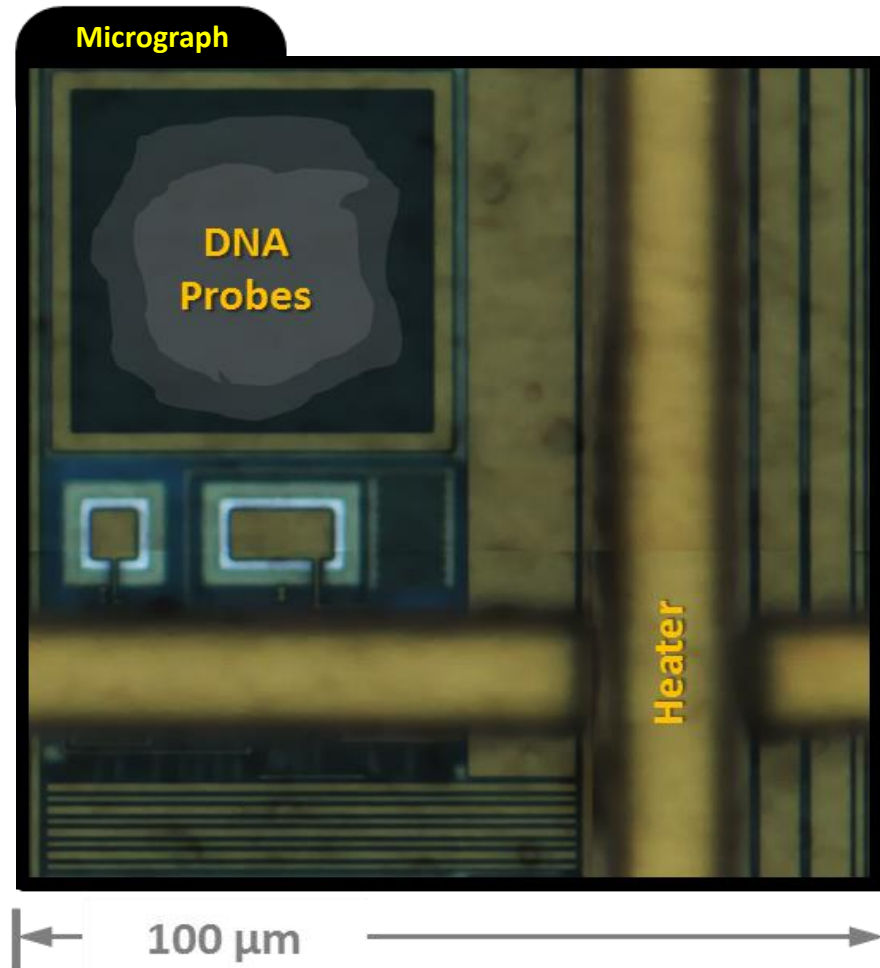
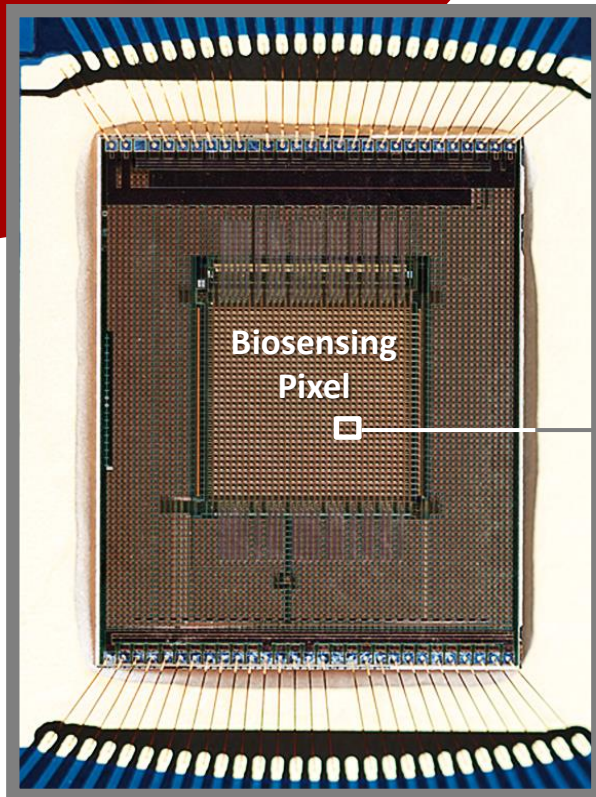
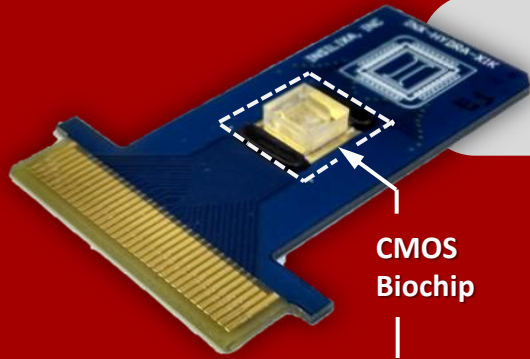
HYDRA-1K: Integrated Pixels

A programmable +90dB dynamic range photo-sensor and thermo-cycler are integrated in every pixel



HYDRA-1K: Integrated Pixels

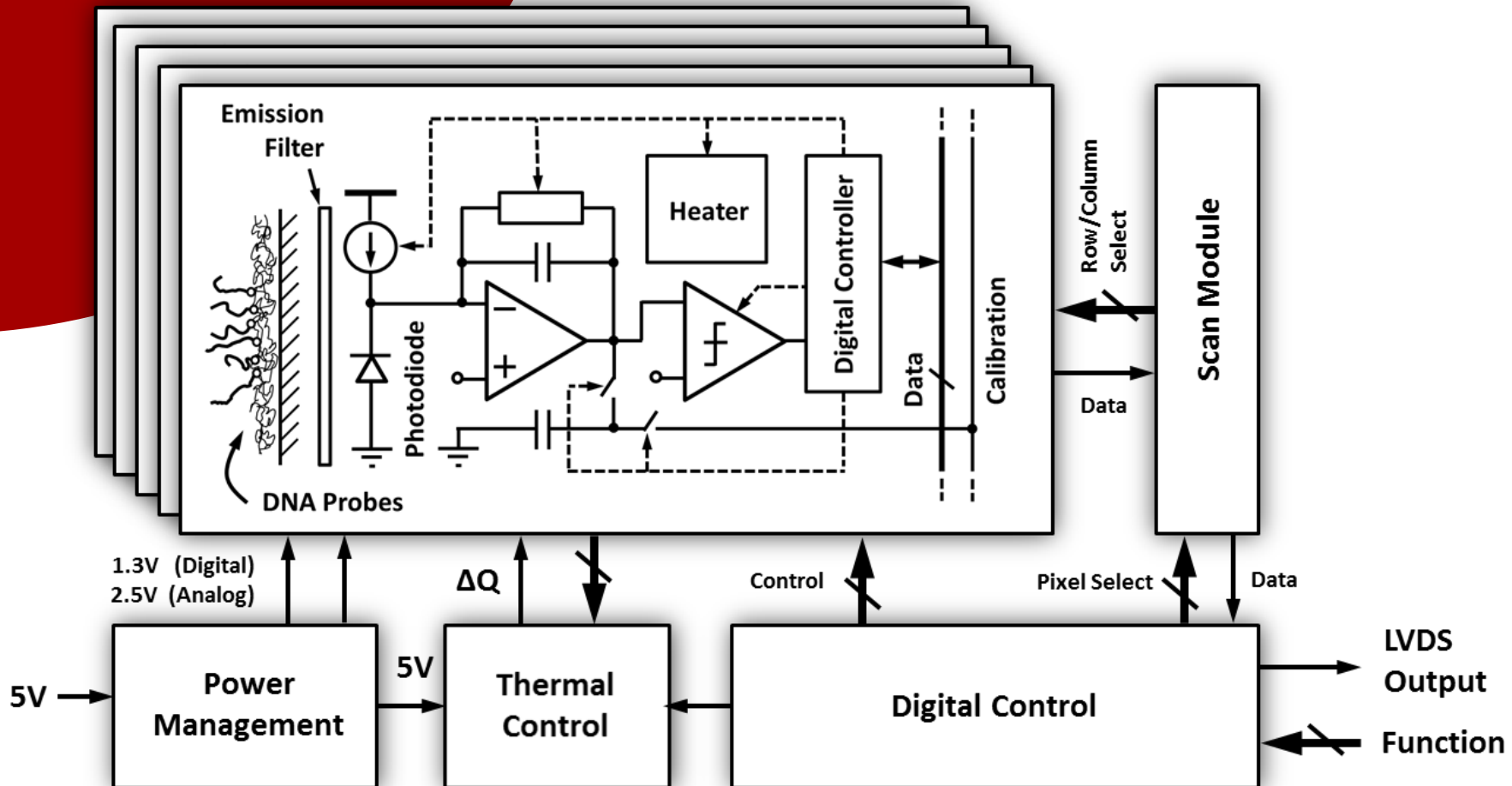
A programmable +90dB dynamic range photo-sensor and thermo-cycler are integrated in every pixel



HYDRA-1K: Chip Architecture

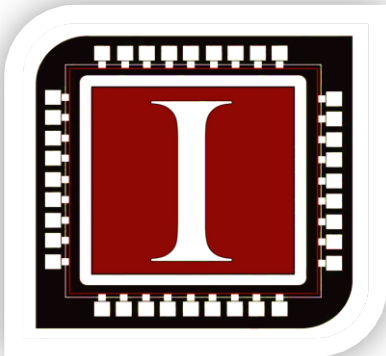
The biochip includes the 1024-element biosensor array, a 24-bit $\Sigma\Delta$ data converter, on-chip thermal controller, and power management system

1024 Pixels



Chip Performance/ Characteristics

TECHNOLOGY	IBM 6RF (0.25 μm)
ARRAY	32 x 32 (1008 Biosens. + 16 Temp. Sens.)
BIOSENSING PIXELS	Fluorescence ($\lambda \sim [450 \text{ nm}, 700 \text{ nm}]$)
DETECTION DYNAMIC RANGE	145 dB ($I_{\text{ph}} \sim [0.5 \text{ fA}, 10 \text{ nA}]$)
SPEED	0.1-50 frames/sec
RESOLUTION	>24 Bit
POWER CONSUMPTION	112 mW ($I_{\text{C}} = 45 \text{ mA @ } 2.5 \text{ V}$)
HEATING/COOLING RATES	(+4/-4) $^{\circ}\text{C/sec}$
TEMPERATURE CONTROL ACCURACY	0.25 $^{\circ}\text{C/sec}$
COST (INCLUDING ASSEMBLY)	\$7-\$15*



EXAMPLE

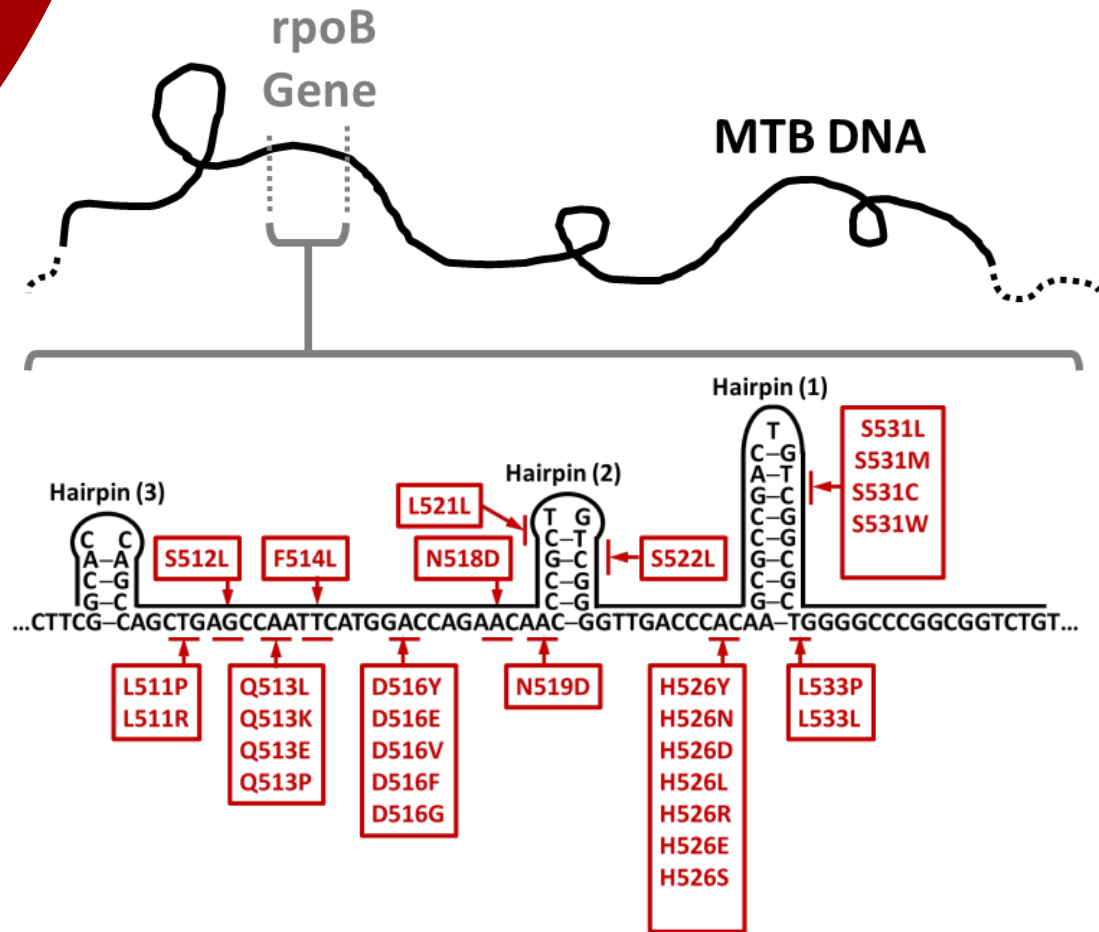
Mycobacterium Tuberculosis (MTB) detection

Detecting Drug Resistant TB

TB bacterium has a very specific region which includes mutations that are result in antibiotic resistivity



*Mycobacterium
Tuberculosis (MTB)*

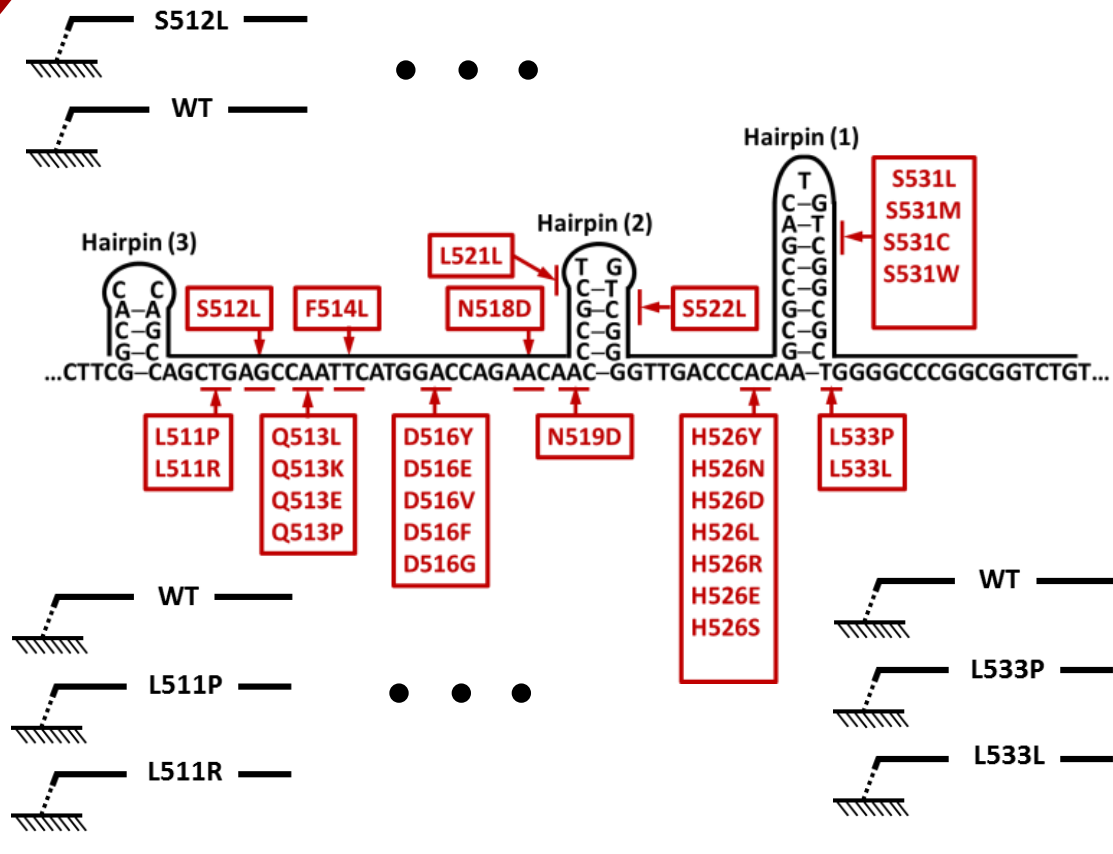
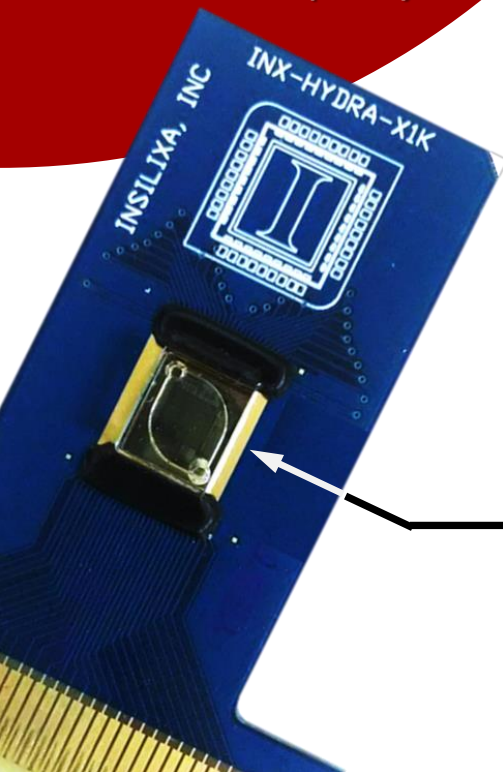


30 mutations within the *rpoB* gene that cause resistivity to Rifampicin

Probe Design Procedure

DNA probes are design to capture the wild-type and the mutant strains for every possible mutation

*Mycobacterium
Tuberculosis (MTB)*



Probe Location within Array

L511P (W)	Control (2)	N519D (W)	H526D (M1)	H526L (M-L)	L533P (W)	S512L (W)	N518D (W)	H526N (M1)	H526N (M-L)	S531L (W)	Control (1)	D516Y (W)	Control (3-H)	H526L (M)	Control (2-Cy)
L511P (M)	F514L (W)	N519D (M)	H526D (M2)	H526R (M-L)	L533P (M)	S512L (M)	N518D (M)	H526N (M2)	H526D (M-L)	S531L (M1)	Q513L (W)	D516Y (M1)	L533P (W)	H526R (W)	H526E (M-L)
L511R (M)	F514L (M)	L521L (W)	H526L (M)	H526E (M-L)	L533L (M)	F514L (W)	N519D (W)	H526D (M1)	H526L (M-L)	S531L (M2)	Q513L (M)	D516Y (M2)	L533P (M)	H526R (M)	H526S (M-L)
S512L (W)	D516Y (W)	L521L (M)	H526R (W)	H526S (M-L)	Control (3-H)	F514L (M)	N519D (M)	H526D (M2)	H526R (M-L)	S531M (W)	Q513K (M)	D516E (M)	L533L (M)	H526E (W)	S531L (W)
S512L (M)	D516Y (M1)	S522L (W)	H526R (M)	S531L (W)	L511P (W)	D516Y (W)	L521L (W)	H526L (M)	H526E (M-L)	S531M (M)	Q513E (M)	D516V (M)	H526Y (W)	H526E (M)	S531L (M1)
Q513L (W)	D516Y (M2)	S522L (M)	H526E (W)	S531L (M1)	L511P (M)	D516Y (M1)	L521L (M)	H526R (W)	H526S (M-L)	S531C (M)	Q513P (M)	D516F (M)	H526Y (M1)	H526S (M)	S531L (M2)
Q513L (M)	D516E (M)	H526Y (W)	H526E (M)	S531L (M2)	L511R (M)	D516Y (M2)	L522L (W)	H526R (M)	L533P (W)	S531W (M1)	F514L (W)	D516G (M)	H526Y (M2)	H526Y (W-L)	S531M (W)
Q513K (M)	D516V (M)	H526Y (M1)	H526S (M)	S531M (W)	Q513L (W)	D516E (M)	S522L (M)	H526E (W)	L533P (M)	S531W (M2)	F514L (M)	N519D (W)	H526Y (M3)	H526Y (M-L)	S531M (M)
Q513E (M)	D516F (M)	H526Y (M2)	H526Y (W-L)	S531M (M)	Q513L (M)	D516V (M)	S526Y (W)	H526E (M)	L533L (M)	L511P (W)	N518D (W)	N519D (M)	H526N (M1)	H526N (M-L)	S531C (M)
Q513P (M)	D516G (M)	H526Y (M3)	H526Y (M-L)	S531C (M)	Q513K (M)	D516F (M)	H526Y (M1)	H526S (M)	S512L (W)	L511P (M)	N518D (M)	S522L (W)	H526N (M2)	H526D (M-L)	S531W (M1)
Control (1)	N518D (W)	H526N (M1)	H526N (M-L)	S531W (M1)	Q513E (M)	D516G (M)	H526Y (M2)	H526Y (W-L)	S512L (M)	L511R (M)	L521L (W)	S522L (M)	H526D (M1)	H526L (M-L)	S531W (M2)
Control (1-Cy)	N518D (M)	H526N (M2)	H526D (M-L)	S531W (M2)	Q513P (M)	Control (2)	H526Y (M3)	H526Y (M-L)	Control (1)	Control (3-H)	L521L (M)	Control (2)	H526D (M2)	H526R (M-L)	Control (1-Cy)



**Wild-Type
Probe**



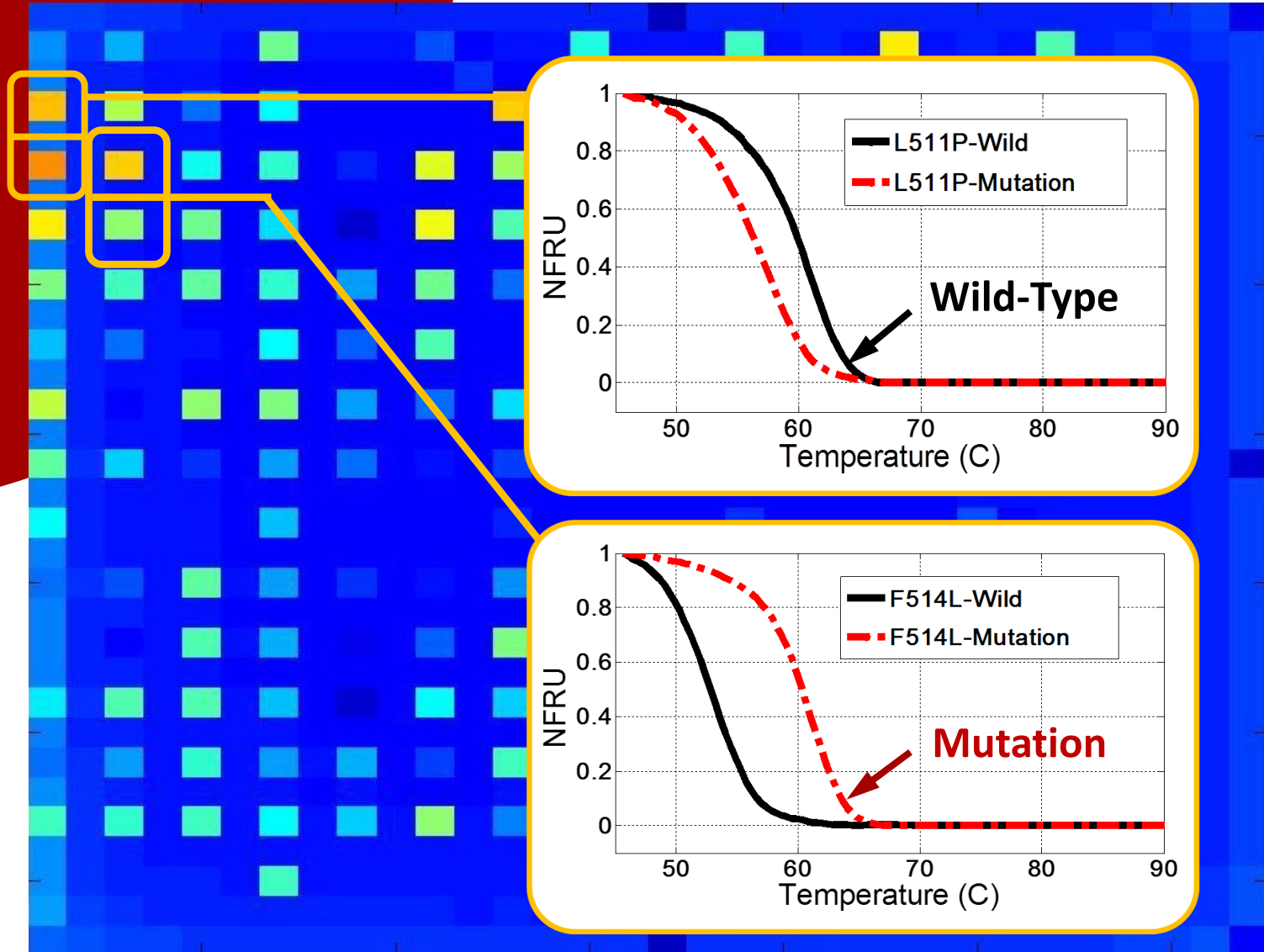
**Mutated Strain
Probe**



Controls



Capturing and DNA Melt-Curve Analysis



Example Results

Mutation	Wild-Type TB		Strain-1		Strain-2		Strain-3	
	Actual	Meas.	Actual	Meas.	Actual	Meas.	Actual	Meas.
L511P	CTG	CTG	C <u>C</u> G	C <u>C</u> G	CTG	CTG	CTG	CTG
Q513L	CAA	CAA	CAA	CAA	C <u>T</u> A	C <u>T</u> A	CAA	CAA
F514L	TTC	TTC	TTC	TTC	TTC	TTC	<u>C</u> TC	<u>C</u> TC
D516F	GAC	GAC	<u>T</u> TC	<u>T</u> TC	GAC	GAC	GAC	GAC
N518D	AAC	AAC	AAC	No Call	<u>G</u> AC	<u>G</u> AC	AAC	AAC
L521L	CTG	CTG	CTG	CTG	CTG	CTG	<u>I</u> TG	<u>I</u> TG
H526Y	CAC	CAC	<u>I</u> AC	<u>I</u> AC	CAC	CAC	CAC	CAC
S531L	TCG	TCG	TCG	TCG	<u>T</u> IG	<u>T</u> IG	TCG	TCG
L533P	CTG	CTG	CTG	CTG	CTG	CTG	<u>I</u> TG	<u>I</u> TG

Sensitivity > 50 Copies-per-Sample
Strain Detection Success Rate= 97.22%

Comparison

TECHNOLOGY	PCR	DNA ARRAYS	DNA SEQUENCING	HYDRA-1K
Instrument (Setup) Price	\$20K -50K	\$25K-75K	\$100K-\$700K	~\$250
Price per Test	\$80-\$400	\$200-\$1000	\$2.5K-\$10K	\$7-\$15*
Max DNA Targets	6-20	20-1000	+1M	1000
Detection Accuracy	High	Low	Medium	High
Test Time	2-3 hours	6-12 Hours	> 1 Day	1 Hour
Fully- Automated	Yes	No	No	Yes
Portable	No	No	No	Yes
PoC Compatible	Yes	No	No	Yes

Conclusion

- **Molecular diagnostics (MDx), including DNA analysis, will become a critical part of future healthcare**
- **Today, creating mass-deployable MDx platforms is of great importance**
- **CMOS biochips offer a possible solution for low-cost, high-performance, and versatile point-of-care MDx**
- **InSilixa is commercializing HYDRA-1k platform, a CMOS biochips product for DNA analysis**

Small DNA Differences Matter



**Albert Einstein
(1879-1955)**

—



**Bobo the Chimp
(1995-Now)**

**= 1.5% DNA
Difference**